

Liver Biopsy in Cirrhotic Patients

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Liver biopsy remains an important tool for the evaluation of patients with hepatic disease. However, clinicians utilize a variety of biopsy techniques including automated cutting needle devices, manual cutting needles, and aspiration needles. Using a large study cohort of patients with advanced fibrosis/cirrhosis we sought to evaluate practices and outcomes of the biopsy technique used by study investigators across the United States. All biopsy samples were from patients with suspected advanced fibrosis or cirrhosis because of hepatitis C virus (HCV) infection. Individual study investigators were permitted to use any biopsy technique. Biopsy specimens were centrally evaluated for tissue adequacy and fragmentation, and were histologically scored using accepted criteria. We evaluated a total of 923 liver biopsy specimens from 502 patients performed at 62 clinical sites. The average duration of HCV infection was 27.9 ± 0.46 yr. Automated cutting needles were significantly more likely to provide adequate specimens for evaluation than aspiration needles ($P < 0.005$). Automated cutting needles produced significantly longer biopsies than other techniques ($P < 0.05$), except for a limited number of cases in which a surgical wedge biopsy was obtained. Tissue fragmentation was observed in 39.2% of liver biopsies obtained using an aspiration technique, but in only 4.7% of samples collected using an automated cutting needle ($P < 0.001$). We conclude that automated cutting needles provide superior liver biopsy specimens compared with aspiration techniques in subjects with advanced fibrosis/cirrhosis. No specific safety issues attributable to a particular biopsy method were identified.

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INTRODUCTION

Liver biopsy represents a key modality in the diagnosis and staging of liver disease. Although first described by Erlich in the late 1800s, liver biopsy did not achieve broad acceptance and use until the 1950s. There has been an evolution in biopsy techniques and methods over the years, but few efforts to characterize differences in biopsy quality. Liver biopsy may be performed using one of four broadly defined methods. These include open (surgical) biopsy, laparoscopic biopsy, percutaneous biopsy, and transvascular intrahepatic biopsy. By far, most liver biopsies are obtained using a percutaneous technique. Percutaneous biopsy may be performed using a suction-type needle (Menghini, Jamshidi, Klatskin) or a cutting needle (Tru-Cut, Vim Silverman). More recent, but increasingly popular, variants of the cutting needles are those with a spring-loaded mechanism that both advances the needle and protects the biopsy section with a sheath that closes over the cutting edge. Biopsy needles vary in gauge and the length of the sample that can be obtained. For many years percutaneous biopsy was performed after site selection was made by a clinician based upon percussion, palpation, and experience. In recent years, imaging modalities including ultrasound and CT have been used as an adjunct in biopsy site selection.

There are limited data regarding biopsy quality that can guide clinicians in the choice of techniques. Colombo *et al.*

noted that the Tru-Cut needle was superior to the Menghini needle in patients with cirrhosis, because of higher rates of tissue fragmentation when the suction-type needle was used (1). Most literature regarding percutaneous liver biopsy has focused on safety rather than quality. For this reason, we sought to determine if biopsy method affected the ability of a skilled hepatopathologist to evaluate liver histology. To this end we analyzed data obtained from a prospective, randomized trial of gamma interferon in subjects with advanced fibrosis and cirrhosis.

METHODS

Liver biopsy specimens were obtained during the performance of a phase II, double-blind randomized placebo controlled multicenter trial designed to determine the safety and efficacy of interferon-gamma 1b monotherapy in patients with hepatitis C virus (HCV)-associated severe liver fibrosis or cirrhosis (Ishak Stages 4 to 6, Metavir F3 or F4). The primary objective was to evaluate the proportion of patients showing a reduction of one or more fibrosis points using the Ishak staging system. The biopsies were collected at 62 clinical sites distributed across the United States. The results of this trial have been described (2). Inclusion criteria permitted enrollment of adult men or women with chronic HCV infection and a willingness to undergo paired liver biopsy at the

beginning and end of therapy. Patients with platelet counts less than 60,000/mm³ were excluded unless the investigator performed a transjugular biopsy or other method deemed to be safe. Subjects with other forms of liver disease were excluded.

The investigators at individual sites were permitted to choose the approach and type of liver biopsy performed, but the sponsor advised that specimens should be at least 20 mm in length, to ensure adequate sampling for evaluation of fibrosis. Biopsy specimens were evaluated at the central pathology laboratory at the Armed Forces Institute of Pathology (AFIP). Sites could submit the specimen in formalin fixative (56%), processed and embedded in a paraffin block (29%), or as unstained slides sectioned from a block (15%). At the time of biopsy, the investigators provided detailed information regarding the liver biopsy including approach (percutaneous, surgical, transvenous), method (aspiration, automated gun, other), and needle type (Jamshidi, Menghini, Tru-Cut, etc.). They also recorded whether ultrasound guidance was used and the number of passes of the needle into the liver. All samples were evaluated using hematoxylin and eosin, Masson trichrome, and Sirius red stains.

A single pathologist (ZDG) blinded to treatment and order evaluated all samples. All specimens were graded for specimen quality on a 3-point scale that classified samples as adequate, marginally adequate, or inadequate for the purpose of the study. Adequate was defined as the presence of at least six portal tracts and absence of fragmentation that would limit assessment of the biopsy stage. Marginally adequate samples had at least three portal areas. Fragmented samples were noted. Total length was measured in the stained sections. Specimens with multiple fragments were evaluated for length as the sum of the fragments in their longest aspect. A computer-generated area of the biopsy was obtained by digital imaging.

Complications of biopsy were prospectively reported on the case report forms designed to capture adverse events reported during the conduct of the study. The study was performed in accordance with the principles governing ethics in medical research. All study sites had Institutional Review Board approval to conduct the study and all patients provided informed consent.

Statistical evaluation was performed using Statistix 7.0 software. Continuous variables were evaluated using Student's *t*-test or analysis of variance (ANOVA) techniques. Categorical measures were evaluated using appropriate non-parametric procedures including Fisher's exact and χ^2 tests. The weighted kappa statistic was utilized to evaluate intraobserver variability in fibrosis staging.

RESULTS

A total of 502 subjects were enrolled in the study. For the purpose of this analysis, all pretreatment and posttreatment

biopsies (when available) were evaluated, without regard to treatment arm (interferon gamma-1b vs placebo). Demographics of the study population are shown in Table 1. The median age of patients at study entry was 51 yr (range 34–76 yr). Sixty-nine percent were male. Slightly over 70% were Caucasian. Data on a total of 923 liver biopsies are reported. This includes a pretreatment biopsy in all patients and a post-treatment biopsy in 421 subjects.

Evaluation of Liver Biopsies

The distribution of biopsy approaches was evaluated among 923 total biopsies performed. A total of 783 (88%) liver biopsies were performed by a percutaneous approach, while 52 (6%) were by a transvenous approach and 53 (6%) were laparoscopic. Investigators failed to report the approach taken in 35 cases. The methods used for the biopsy were reported in 840 cases. An aspiration needle was used in 250 subjects (30%), while 530 (63%) cases were biopsied with an automated biopsy gun device. The remaining 60 (7%) cases were obtained by another technique, or were listed as unknown (*N* = 30). Investigators failed to report the biopsy method in the remaining 53 cases. Specific needle type was requested from the investigators, but many reported using an automated gun device and then indicated the needle type was a Tru-Cut, suggesting this term has been generalized by many clinicians to mean a cutting needle as opposed to an aspiration or suction-type biopsy needle. Multiple brands of automated biopsy needles were also listed. Needle sizes ranged from 15 to 20 G. Nearly 72% of biopsies were performed using ultrasound assistance for identification of biopsy site.

All liver biopsies were visually evaluated for fragmentation, adequacy, and measured to determine length. Cross-sectional areas were also calculated. Figure 1 demonstrates the relationship between method of liver biopsy and presence or absence of fragmentation. Fragmentation was observed in 39.2% of liver biopsies obtained using an aspiration technique, but in only 4.7% of samples collected using an automated cutting needle (*P* < 0.001).

Adequacy of the biopsy specimen for histologic evaluation as determined by the study pathologist was also evaluated. Use of the automated cutting needles was associated

Table 1. Demographic Features of Study Population

Number of liver biopsies (N)	923
Age (median) and range in years	51 (34–76)
Gender (%)	
Male	68.9
Female	31.1
Race (%)	
White	70.5
Native American	0.2
African American	10.4
Asian-Pacific Islander	1.4
Hispanic/Latino	16.5
Other	1.0
Estimated duration of HCV infection in years \pm standard error of mean	27.9 \pm 0.46

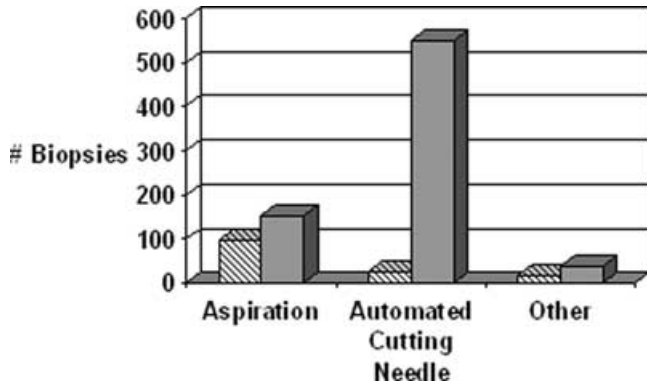


Figure 1. Presence of fragmentation of liver tissue by the method of biopsy. Striped line indicates the presence of fragmentation, gray line indicates no fragmentation.

with a qualitative evaluation of adequate in 553 biopsies (93%), marginal in 36 biopsies (6%), and inadequate in 6 (1%). In contrast, aspiration techniques led to 207 (83%) biopsies judged adequate, 37 marginal (15%), and 6 inadequate (2%). Statistical comparison between aspiration methods and automated cutting needle techniques reveals that aspiration methods were more likely to be judged inadequate ($P = 0.005$). Because the study required an adequate/marginally adequate biopsy for enrollment, all patients had an adequate first biopsy, and the inadequate samples were among the follow-up biopsies at completion of therapy.

Mean biopsy length was 19.17 mm (standard error of mean [SEM] 0.31) across the 886 biopsies evaluated. The distribution of biopsy length is shown in Figure 2. Mean length was 17.47 mm (SEM 0.52) for aspiration biopsies, 20.26 mm (SEM 0.37) for automated cutting needle biopsies, and 16.75 mm for other biopsy types. ANOVA indicated that automated cutting needles produced significantly longer biopsies than other types ($P < 0.05$). However, the two largest biopsies (>90 mm) were derived from surgical wedge biopsies.

Biopsy length is closely associated with its quality assessment. The mean length of specimens graded as adequate

was 20.18 mm, but marginally adequate specimens had a mean length of 8.22 mm ($P < 0.05$). Inadequate specimens were even smaller (7.18 mm). Significantly more passes into the liver were performed using the automated cutting needles *versus* the aspiration needles. Nearly 61% of aspiration biopsies were completed in one pass. In contrast, only 34% of automated gun biopsies reported only one pass ($P < 0.001$), while the majority (45%) required two passes, and 20% reported that three passes of the needle were performed. The two-dimensional surface area of each biopsy was also evaluated. The mean surface area was 13.4 mm² (SEM 0.56 mm²). There was a wide range of surface areas in the sample set ranging from 0.66 to 368 mm² (wedge biopsy). Univariate analysis showed that surface area was highly correlated with length, number of passes, use of ultrasound, and method of biopsy, but not with age, gender, or degree of fibrosis. Linear regression modeling (unweighted least-square model) confirmed the significance of the univariate factors in the following order: length > ultrasound > passes > method. Length was more highly correlated with assessment of quality than surface area in a regression model.

Reproducibility of biopsy interpretation was evaluated using 60 biopsies from the study and then retested on a second set of 60 biopsies. In both sets, Ishak fibrosis scores (0–6) were identical in 72% and ± 1 stage in 100%, with a weighted kappa statistic of 0.71.

In terms of pathologic assessment, 752 (81.7%) biopsies were interpreted as showing cirrhosis, while 168 (18.3%) had bridging fibrosis. The Ishak score, which permits more gradations in fibrosis/cirrhosis, rated 504 biopsies (54.8%) as established cirrhosis, stage 6; 248 (27.0%) as incomplete cirrhosis, stage 5; 140 (15.2%) as marked bridging fibrosis, stage 4; and 28 (3.1%) as occasional bridging fibrosis, stage 3. Overall, there was very little change in fibrosis scores among patients with paired biopsies (Fig. 3). Among evaluable pairs, only 63 subjects improved and 62 worsened with no differences between treatment groups, so most of the changes probably represent sampling variability of needle biopsies.

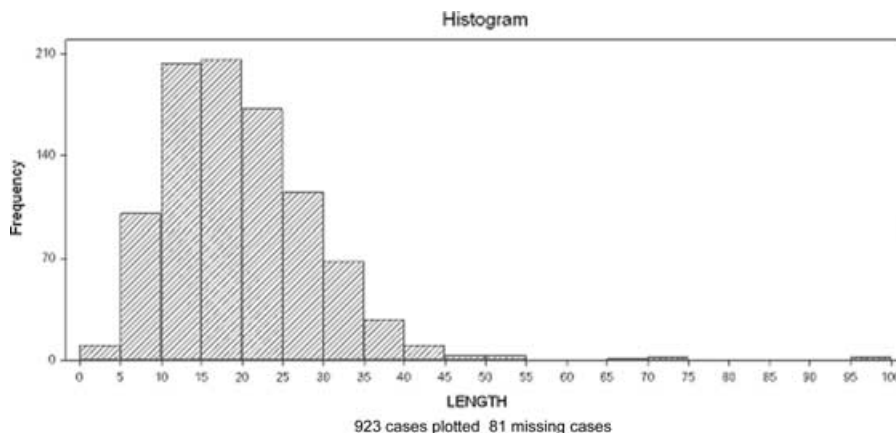


Figure 2. Histogram of liver biopsy length.

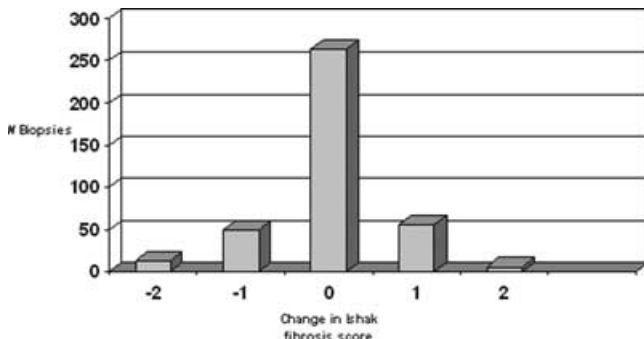


Figure 3. Ishak fibrosis score change among evaluable paired biopsies.

Among patients whose biopsy appeared to improve, the cases were equally distributed among those sampled using an aspiration technique *versus* those using a cutting needle technique. However, among those noted to have fragmented samples, 75% were biopsied by an aspiration method *versus* 25% sampled with an automated gun device ($P = 0.1$). Within this group whose Ishak fibrosis scores declined there was a significant rate of discordance in method between the first and second biopsy. Ten subjects biopsied the first time using an aspiration method were biopsied the second time using an automated cutting needle, suggesting that fragmentation of specimen led to a spurious diagnosis of established cirrhosis while nonfragmented specimens from the same patients were correctly interpreted as having lower fibrosis stages. Only one subject had discordant biopsy methods in the reverse order.

There were no serious adverse events associated with liver biopsy in any subject and no procedure-related mortality. This includes hospitalization for observation, blood transfusion, or surgery. Postprocedure pain that did not result in classification as a severe adverse event was not captured in the study database.

DISCUSSION

This study describes 923 liver biopsies performed in patients with advanced liver fibrosis as a result of chronic hepatitis C infection. Prior studies of liver biopsy techniques have included patients with a broad spectrum of diseases and degrees of hepatic scarring. Because all patients in the present study had HCV infection without tumor but with advanced fibrosis, there was significant homogeneity in the study population, permitting us to assess differences in methods of biopsy in the context of a multicenter trial. Furthermore, the use of paired biopsies in the setting of an ineffective drug treatment and placebo arm permits assessment of liver biopsy tissue variability and sampling error.

Interestingly, most of the biopsies performed in this study were performed by an automated cutting needle device. These devices represent the second generation of the older manual Tru-Cut needles. Their use has been evaluated previously in terms of safety and efficacy but not with an internal comparison group of aspiration (suction) biopsy needles (3–5).

Previous reports indicate that the Tru-Cut needle was significantly better than the Menghini needle in providing tissue judged to be adequate for histological evaluation. Herein, we provide evidence that tissue adequacy is significantly greater using automated cutting needle devices when compared with aspiration devices among subjects with advanced fibrosis. Differences in fragmentation of the samples are a major contributor to classification as an inadequate sample for histological evaluation.

The mean length of biopsies was greater for the automated biopsy gun devices *versus* aspiration needles. However, there was a significantly higher rate of biopsies requiring more than one pass for the automated cutting needles. This was not associated with an apparent increase in the complication rate. Similarly, Maharaj and Bhoora found that multiple passes through a single entry site did not increase the risk of complications (6). In other series, the number of passes into the liver was associated with increased complication risk (7). We can only speculate as to why more passes are performed using the automated cutting needles. It is possible that clinicians performing the biopsy did not feel that the first pass was adequate and that a second pass was required. The total biopsy length possible to obtain varies by needle manufacturer, but several popular products limit the total length of the cutting blade to approximately 2 cm. Alternatively, it is possible that clinician comfort when using this device is so high that two passes were deemed safe and acceptable compared with clinicians who used aspiration needles. The high rate of ultrasound use, which was not mandated by the study criteria, suggests that this procedure has been widely accepted, even though there are limited data to suggest that use of ultrasound improves either efficacy or safety (8, 9). The finding that length rather than area had a higher correlation with grading of quality was somewhat surprising. However, other authors have cited the importance of biopsy length (10). These features were highly correlated however. Rocken *et al.* reported comparison of 20 G liver biopsies to those using a 17 G needle and found no difference in ability to provide histopathologic diagnoses, whereas other studies found larger bore needles to be superior to narrow bore needles, especially in the evaluation of fibrosis (11, 12). Our dataset did not permit direct assessment of needle gauge as a contributor to quality because many investigators failed to provide this information.

The lack of adverse clinical consequences in this group of patients with advanced liver fibrosis is reassuring. Other studies have suggested that presence of tumor, cirrhosis, operator experience, and number of passes may be associated with increased biopsy complications. In a survey of 68,276 biopsies, tumor and cirrhosis were associated with hemoperitoneum (13). Number of passes was a key association with complications in the report of Perrault *et al.*, (7) but as noted above, other studies appear to contradict these findings (6).

While the lack of formal randomization might be perceived as a limitation of this analysis, it may also be construed as an asset in our understanding of real-world practices.

Experienced clinicians were asked to perform biopsies in patients that were suspected of having advanced liver fibrosis. Despite this knowledge, there was no uniform choice made in biopsy method, needle type, or gauge. Therefore, the trial truly mirrors the conflicting beliefs regarding best practice as related to liver biopsy. Another limitation is related to the exclusion of biopsy samples with minimal or moderate fibrosis. It is possible that a comparison of sample adequacy weighted toward early fibrosis stages would discern no difference with regard to needle type. However, we believe that in the absence of recent prior biopsy, and prior to signs and symptoms of cirrhosis, it is difficult for the clinician to know which patients need biopsy performed with an automated cutting needle *versus* an aspiration method. The default should lie with the cutting needle devices.

In summary, we find that HCV-infected patients with advanced fibrosis/cirrhosis can be safely biopsied using automated cutting needle devices. In this population, use of automated cutting needles provided better quality tissue for histological evaluation than aspiration-(suction)-type needles. Clinicians should consider use of automated needles in all patients known or suspected to have advanced fibrosis/cirrhosis.

STUDY HIGHLIGHTS

What Is Current Knowledge

- Liver biopsy remains the gold standard for evaluation of liver fibrosis.
- Liver biopsy may be performed with both automated cutting needles and aspiration (suction) needles and experienced hepatologists/gastroenterologists utilize a variety of biopsy methods in routine practice.

What Is New Here

- Automated cutting needles provide superior quality liver biopsies in subjects with advanced fibrosis or cirrhosis.
- No significant safety issues were identified in the biopsied population despite the presence of advanced liver disease.

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CONFLICT OF INTEREST

Guarantor of the article: Kenneth E. Sherman

Specific author contributions: Kenneth E. Sherman—Data analysis, manuscript preparation; Zachary D. Goodman—Biopsy review, data analysis, manuscript review; Sara T. Sullivan—Parent study design, manuscript review; Sima Faris-Young—Parent study design, manuscript review

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